is loaded into a disposable mini column and the flow thru (depleted lysate) is collected and saved for later evaluation. The resin is washed twice with lysis buffer that has been adjusted to pH 7.0; the volume of each of these washes is equal to the original volume of cleared lysate. The flow thru of these two washes is also saved for later analysis in western blots to evaluate purification efficiency.

At this point the columns contain relatively purified recombinant proteins which are immobilized by the His tags at their C-terminus. This is an ideal situation for refolding, so the column is moved to a 4° C. room and a series of renaturation buffers with decreasing urea concentrations are passed through the column. The renaturation buffers contain varying amounts of urea in 25 mM Tris pH 7.4, 500 mM NaCl, and 20% glycerol. This buffer is prepared as stock solutions containing 6M, 4M, 2M and 1M urea. Aliquots of these can be easily mixed to obtain 5M and 3M urea concentrations thus providing a descending series of urea concentrations in 1 M steps. One volume (the original lysate volume) of 6M buffer is passed through the column, followed by one volume of 5M buffer, continuing on to the 1 M buffer which is repeated once to ensure equilibration of the column at 1M urea. At this point the refolded proteins are eluted in 8 fractions of $1/10^{th}$ original volume using 1M urea, 25 mM Tris pH 7.4, 500 mM NaCl, 20% glycerol containing 250 mM imidazole. The imidazole disrupts the Nickel ion-His tag interaction, thereby releasing the protein from the column.

Western blots are used to evaluate the amount of His tagged protein in the depleted lysate, the two washes, and the eluted fractions. If there is an abundance of recombinant protein in the depleted lysate and/or washes it is possible to repeat the process and "scavenge" more protein. Eluate fractions that contain the protein of interest are pooled and then concentrated and exchanged into storage buffer (20 mM Tris pH 7.4, 10 mM NaCl, 10% glycerol) using centricon centrifugal ultrafiltration devices (Millipore). The enzyme preparations are then aliquoted and frozen at −80° C. for use in activity assays.

In various embodiments of this invention, the cellulose degrading enzymes, related proteins and systems containing thereof, of this invention, for example including one or more enzymes or cellulose-binding proteins, have a number of uses. Many possible uses of the cellulases of the present invention are the same as described for other cellulases in the paper "Cellulases and related enzymes in biotechnology" by M. K. Bhat (Biotechnical Advances 18 (2000) 355-383), the subject matter of which is hereby incorporated by reference in its entirety. For examples, the cellulases and systems thereof of this invention can be utilized in food, beer, wine, animal feeds, textile production and laundering, pulp and paper industry, and agricultural industries.

In one embodiment, these systems can be used to degrade cellulose to produce short chain peptides for use in medicine.

In other embodiments, these systems are used to break down cellulose in the extraction and/or clarification of fruit and vegetable juices, in the production and preservation of fruit nectars and purees, in altering the texture, flavor and other sensory properties of food, in the extraction of olive oil, in improving the quality of bakery products, in brewing beer and making wine, in preparing monogastic and ruminant feeds, in textile and laundry technologies including "fading" denim material, defribillation of lyocell, washing garments and the like, preparing paper and pulp products, and in agricultural uses.

In some embodiments of this invention, cellulose may be used to absorb environmental pollutants and waste spills. The cellulose may then be degraded by the cellulose degrading systems of the present invention. Bacteria that can metabolize environmental pollutants and can degrade cellulose may be used in bioreactors that degrade toxic materials. Such a bioreactor would be advantageous since there would be no need to add additional nutrients to maintain the bacteria they would use cellulose as a carbon source.

In some embodiments of this invention, cellulose degrading enzyme systems can be supplied in dry form, in buffers, as pastes, paints, micelles, etc. Cellulose degrading enzyme systems can also comprise additional components such as metal ions, chelators, detergents, organic ions, inorganic ions, additional proteins such as biotin and albumin.

In some embodiments of this invention, the cellulose degrading systems of this invention could be applied directly to the cellulose material. For example, a system containing one, some or all of the compounds listed in FIGS. **4-11** could be directly applied to a plant or other cellulose containing item such that the system would degrade the plant or other cellulose containing item. As another example, 2-40 could be grown on the plant or other cellulose containing item, which would allow the 2-40 to produce the compounds listed in FIGS. **4-11** in order to degrade the cellulose containing item as the 2-40 grows. An advantage of using the 2-40 or systems of this invention is that the degradation of the cellulose containing plant or item can be conducted in a marine environment, for example under water.

It is one aspect of the present invention to provide a nucleotide sequence that has a homology selected from 100%, 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% to any of the sequences of the compounds listed in FIGS. **4-11**

The present invention also covers replacement of between 1 and 20 nucleotides of any of the sequences of the compounds listed in FIGS. **4-11** with non-natural or non-standard nucleotides for example phosphorothioate, deoxyinosine, deoxyuridine, isocytosine, isoguanosine, ribonucleic acids including 2-0-methyl, and replacement of the phosphodiester backbone with, for example, alkyl chains, aryl groups, and protein nucleic acid (PNA).

It is another aspect of some embodiments of this invention to provide a nucleotide sequence that hybridizes to any one of the sequences of the compounds listed in FIGS. **4-11** under stringency condition of 1×SSC, 2×SSC, 3×SSC, 4×SSC, 5×SSC, 6×SSC, 7×SSC, 8×SSC, 9×SSC, or 10×SSC.

The scope of this invention covers natural and non-natural alleles of any one of the sequences of the compounds listed in FIGS. **4-11**. In some embodiments of this invention, alleles of any one of any one of the sequences of the compounds listed in FIGS. **4-11** can comprise replacement of one, two, three, four, or five naturally occurring amino acids with similarly charged, shaped, sized, or situated amino acids (conservative substitutions). The present invention also covers non-natural or nonstandard amino acids for example selenocysteine, pyrrolysine, 4-hydroxyproline, 5-hydroxylysine, phosphoserine, phosphotyrosine, and the D-isomers of the 20 standard amino acids.

It is to be understood that while the invention has been described above using specific embodiments, the description and examples are intended to illustrate the structural and functional principles of the present invention and are not intended to limit the scope of the invention. On the contrary, the present invention is intended to encompass all modifications, alterations, and substitutions within the spirit and scope of the appended claims.

REFERENCES CITED

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